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The physiology of the sheep blow-fly *Lucilia sericata* Meig. (Diptera).
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THE PHYSIOLOGY OF THE SHEEP BLOW-FLY *LUCILIA SERICATA* MEIG. (DIPTERA)

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[Read 4th March, 1936.]

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I. Introduction.

In view of the great economic importance of the sheep blow-fly, *Lucilia sericata*, as shown by the wealth of publications appearing from many parts of the world, it is felt that a review of the physiology of this insect might be of value; especially since physiological work is becoming recognised as an integral part of any investigation on insect pests. Not that *L. sericata* is entirely a pest—its carnivorous habit has been utilised in clearing away dead tissues in chronic osteomyelitis, and it is highly thought of in this connection in the United States of America (Robinson, 1933).

II. The Egg.

Evans (1934) has studied some of the factors governing the loss of water from the egg. The chorion appears to be the chief morphological feature governing the rate of loss of water. Normal eggs were shown to have a 100 per cent. survival and development at 70 per cent. relative humidity and above, while if the chorion is slightly split only a few eggs developed at 80 per cent. relative humidity. If the chorion is completely removed, no eggs developed at 90 per cent. relative humidity. Controls with the chorion split or removed showed 100 per cent. development in a saturated atmosphere. The vitelline membrane also retards the rate of loss of water.

Evans traced a linear relationship between humidity and the length of development and between humidity and the amount of water lost over short periods of time, uncomplicated by morphological changes. Also the amount of water lost varies directly as the amount of water initially present which is usually between 70 and 80 per cent. The amount of water lost at a constant saturation deficiency varies with the temperature, an increase in temperature from 10° C. to 22° C. doubling the amount lost at 6 and 10 mm. sat. def. The three important variables which may prevent the eggs ultimately hatching are

temperature, humidity and time of exposure. It was found, considering the conditions of temperature, humidity and time which prevent hatching, that these three variables can be related to each other in a simple and direct manner by means of the formula

$$y = m(x \times z) + c$$

where y = temperature, ° C., x = saturation deficiency in mm. Hg and z = time of exposure.

III. The Larva.

The physiology of digestion of larvae in carrion and the relation of the microflora to the digestion have been studied in detail by Hobson (1931*b*, 1932*a*, *b*, and *c*, 1935*a*), while Mackerras and Freney (1933) have published some observations on the food of larvae on living sheep. The general anatomy of the alimentary canal is well known and need not be described here. There is one point, however, which merits attention. The midgut is long and convoluted, and three regions can be distinguished—an anterior, a middle and a posterior (Hobson, 1931*b*). The anterior and posterior regions are comparatively thick and contain liquid food. The middle region is constricted at both ends and in a fed larva is distended by the convoluted peritrophic membrane filled with nearly solid food. The histology of these regions is interesting; the anterior and posterior regions in fed larvae possess vacuolated cells while the middle region has non-vacuolated, deeply staining cells. Vacuolation is due to the presence of fat which is probably a temporary storage product. In starved larvae the cells of all three regions are similar, being vacuolated and deeply staining.

The distribution of digestive enzymes is given in Table I. The + signs indicate roughly the relative activity of the various enzymes, the sign ± representing definite but very weak activity. These results are such as might be expected in a flesh-feeding insect. Enzymes acting upon carbohydrates are absent except for a weak amylase in the salivary glands; proteolytic and lipolytic enzymes are active in the midgut, the former being present also in the excreta. There is no doubt that the tryptase in the excreta is derived from the midgut. The proteolytic enzymes are concentrated in the anterior and posterior sections of the midgut. Similar results have been obtained on larvae reared aseptically, so the enzymes present are actually secreted by the larvae and are not produced by bacteria. Mackerras and Freney (1933) record a peptase in the excreta of third-instar larvae.

Table I.

Distribution of digestive enzymes in larvae of *L. sericata*.

Enzyme.	Salivary glands.	Midgut.	Hindgut.	Excreta.
Amylase	±	−	−	?
Invertase	−	−	−	−
Maltase	−	−	−	−
Lactase	−	−	−	−
Tryptase	−	++	±	+
Peptase	−	−	−	−
Peptidase	−	++	?	±
Lipase	?	++	?	?

(Hobson, 1931*b*.)

The hydrogen ion concentration is different in the various divisions of the alimentary tract (Hobson, 1931*b*). The reaction of the crop contents is similar to that of the food. In the anterior region of the midgut, the *p*H varies from 6·5 with fresh neutral gelatine to 7·5 with decomposing meat. The middle region is distinctly acid *p*H 3·2 and the posterior region alkaline *p*H 8·0. The hindgut also has an alkaline reaction *p*H 8·2. The causes of these progressive changes in reaction would appear to be due to the secretion of free phosphoric acid by the cells of the anterior and middle segments of the midgut and to the production of ammonia in the hindgut. The changes of reaction are similar in sterile and non-sterile larvae.

The larvae live chiefly on liquefied food (Hobson, 1931*b*; Guyenot, 1907), but Mackerras and Freney (1933) record particles of liver of irregular shape measuring up to 75 × 50 in the crops of *L. cuprina*, so it would be more correct to say that the food must be in a finely divided state. It is evident that the changes occurring in muscle after death are important factors in the physiology of the larvae feeding upon it. A muscle consists of a network of connective tissue binding together the muscle fibres which form its main bulk (Moran and Smith, 1929). The connective tissue is composed mainly of collagen. A delicate elastic sheath (the sarcolemma) encloses each muscle fibre, whose contents (sarcoplasm) are semi-liquid in consistency and appear to possess a definite arrangement.

Two processes occur during rigor mortis :

(*a*) gelation of the muscle plasma (sarcoplasm);
(*b*) production of an acid which coagulates the protein gel and liberates an acid serum.

Since the acidity of dead muscle appears to prolong rigor mortis and causes the separation of serum, the alkaline reaction of blown meat is likely to be a significant factor in the liquefaction of muscle substance. Experiments on meat and muscle after exposure to ammonia vapour show that the reaction of the food determines the type of food ingested. Acid food is moist whereas, if it is made alkaline by exposure to ammonia vapour until a *p*H of 8·5 is reached, it becomes quite dry to the touch. Table II shows the results obtained

TABLE II.

Nature of food ingested by half-grown, starved larvae from muscle under different conditions.

Medium.	Reaction.	Observations.
Fresh meat (beef).	Acid.	Larvae sucked serum for several hours.
One day old muscle (frog).	Acid.	do.
Ammoniated meat.	Alkaline.	Crops were rapidly filled with a thin jelly which did not dissolve in water.
Ammoniated frog muscle.	Alkaline.	Larvae sucked liquid for 10–15 min.; then a white coagulum began to appear in the crop. After 40 min. feeding the crop contents were an insoluble jelly. In larvae removed from muscle after 10 min. feeding, the crop content remained liquid and did not coagulate.
Meat pressed to remove juice.	Acid.	Larvae sucked juice at first; after 20 min. began to ingest muscle substance, which appeared in the crop as a white coagulum.

(Hobson, 1932*b*.)

on the two types of food. These observations show that larvae only ingest muscle substance when the serum is removed.

The newly emerged larvae feed at first on the fluid present in muscle, but as this becomes alkaline under the influence of bacteria and the protease of their excreta, they attack the muscle substance which is made available through the action of excreted enzymes, protease and collagenase. The newly emerged larvae tend to feed together in bunches; thus enzymatic action on the structural elements of the muscle is concentrated at one or a few points but none or little extra-intestinal digestion occurs as the concentration of enzymes in the ingested food is inappreciable. The presence of a collagenase is probably important in breaking down the connective tissue elements surrounding each muscle fibre. No keratinase is present (Hobson, 1933).

The crop appears to function as a storage organ, since in a starved larva fed on fresh blood the food passes directly into the midgut. Blackening of the blood occurs in the middle segment of the midgut and is probably due to hydrolysis of haemoglobin. Here food is submitted to concentration, as in larvae fed on coloured foods the concentration of the dye is higher in the middle segment than in the anterior segment. The acidity of the middle segment would serve to check any multiplication of ingested bacteria. Tryptase is well adapted for acting in the midgut, since its optimum lies at about *p*H 7·6 and the reaction is approximately *p*H 8·0 in the posterior segment where digestion mainly occurs. Absorption takes place chiefly in the posterior section of the midgut, but some absorption occurs in the anterior region.

Micro-organisms play no part in the digestion of food in the gut. Sterile larvae grow successfully on sterile minced brain. Table III shows that the presence of bacteria improves development, but the difference in rate of growth of sterile and of non-sterile larvae is not marked. The remarkable feature of the intestinal flora is its extreme limitation, consisting chiefly of non-lactose fermenting bacilli in large numbers. The predominance of non-liquefying organisms suggests strongly that the natural flora does not form proteolytic enzymes in the intestine, but that proteolytic forms present in the food probably accelerate the liquefaction of meat.

TABLE III.

Growth of *Lucilia* larvae at 23° C. on heated brain mush in the presence and absence of bacteria.

Age of larvae (hours).	Mean weight per larva in mg.	
	Sterile cultures.	Infected cultures.
0	0·05	0·05
40	1·9	2·8
56	10·1	12·8
80	37·4	51·7
100	56·4	60·6

(Hobson, 1932*a*.)

Although micro-organisms play no part in the digestion of food, their presence in blown meat is distinctly advantageous to developing larvae. On muscle infected from blown meat growth is normal. On muscle infected with a pure culture of *B. coli* growth is almost normal, with a slight lag in the early stages. Aseptic larvae feeding on sterile muscle increase their weight

200 times in 3 days, but later growth is poor and even after 6 days they do not reach full size. Sterile muscle after exposure to ammonia vapour improves the development of aseptic larvae throughout the whole growing period. Thus the main function of micro-organisms is to supply a growth-promoting factor. This substance may be only partly deficient in muscle, since aseptic larvae growing slowly complete more than half their development in 7 days and aseptic larvae growing on ammoniated muscle complete their development in 7 days as opposed to the normal time of 4 days. The factor supplied by the bacteria does not appear to be nucleic acid, vitamin B_1 or B_2, but bears some resemblance to "Factor Y," a heat stable water soluble substance essential for the growth of rats.

Mackerras and Freney (1933) state that, if anything, larval growth is more rapid on the living sheep than on carrion, but it is not clear on what elements, if any, the larvae feed immediately on hatching and before a skin reaction begins. An intense irritation is set up in the skin either mechanically by the jaws of the larvae, or by their secretions, or by the action of the bacteria which they disseminate; most probably all three factors play a part. A serious exudate now appears, which contains coagulable protein, probably albumen and peptones, and forms a suitable food for larval development. Later fresh blood appears and this may be seen in the gut of the larvae.

Occasionally they found small numbers of maggots developing away from the skin surface in faeces-soiled wool. Experiments showed that no development occurs in clean body wool, but complete development occurred in some samples of crutch wool and wool taken from a previously struck area. Sheep from which crutch wool was obtained all showed irritation of the skin, so probably substances other than faecal matter were present. Larvae will survive in fresh sheep's dung, but growth is very slow; one undersized larva pupated, but an adult did not emerge. In wool taken from a previously struck area a "gummy crust" was present; this was obviously the dry exudate from the previous strike and no doubt formed the food of the larvae.

Hobson (1933) has studied the vitamin requirements of larvae grown on blood and, since larvae parasitic on sheep feed on a serous exudate and fresh blood, the results obtained are of great importance. Larvae feeding on non-sterile blood grow very slowly and finally reach full size, but under aseptic conditions no growth occurs. If, however, a yeast preparation, marmite, is added to the blood, normal growth occurs even under aseptic conditions. The results obtained on sterile blood show that larvae resemble the higher animals in their vitamin B requirements. The effect of yeast is due to several substances, and normal growth only occurs if each is present in adequate amount. Experiments with Peters' antineuritic concentrate indicate that one of the larval growth factors is identical with vitamin B. The other growth factors are probably analogous to members of the vitamin B complex and consists of :

(i) a water insoluble growth factor present in muscle and yeast;
(ii) a water soluble heat-stable factor present in autoclaved yeast extract extremely stable to heat and alkali;
(iii) a water soluble heat-stable factor present in autoclaved yeast extract less stable to alkali than (ii).

In the case of larvae feeding on non-sterile blood the site of synthesis of vitamin B appears to be in the food and not in the intestine. No specific organism is involved.

Cholesterol appears to be the only fat soluble growth factor required for growth, Hobson (1935*b* and *c*). Other sterols promote growth but are less active than cholesterol. The larvae grew quite well on a fat-less basal diet with the addition of only cholesterol, so it seems that they can synthesise fat readily. The absence of sterols, besides affecting the growth rate directly also rendered the larvae susceptible to bacterial infection. Natural immunity is attributed to the acid secretion of the midgut, this acidity being dependent on the presence of the cholesterol in the food.

Michelbacher *et al.* (1932) have developed a synthetic medium for studying the nutritional requirements of larvae under sterile conditions. It consists of highly purified casein, yeast, mineral salts, cod-liver oil and cystine. It is suggested that the cystine requirements of the larvae are greater than that of mammals.

Cousin (1932) has studied the changes in respiratory activity during larval growth. The respiratory quotient varies between 0·76 and 0·82, as would be expected on a predominantly protein diet. The intensity of respiration, however, diminishes gradually from hatching until feeding ceases, the oxygen consumption being 8·20 c.c. per gram per hour in very young larvae and 3·15 c.c. in fully grown larvae.

It is well known that blow-fly larvae are negatively phototropic. Ellsworth (1933) shows that only rays of light striking the head are effective in orientation and that removal of the tips of the maxillary lobes causes a definite inability on the part of the larvae to orient themselves. Each larva possesses two pairs of highly specialised photoreceptive organs contained within the bilobed maxillae. Each organ consists of a biconvex lens, a preretinal membrane, a fasciculus of fibrils, retinal cells and a nerve-bundle; it is devoid of pigment cells.

Davies and Hobson (1935) have studied the effect of humidity on the survival of starved larvae of all instars from the point of view of the temperature (37° C.) and humidities existing in the wool of living sheep. First-instar larvae subjected to relative humidities of less than 70 per cent. succumbed in approximately two hours. Young second-instar larvae were more resistant, dying within 2·5 and 3·7 hours at 50 and 70 per cent. relative humidities respectively. Older second-instar larvae survived at 50 per cent. relative humidity for 12 hours. Third-instar larvae withstood still longer exposure to dry conditions. The rapid rise in resistance between the early second and early third instars suggests that storage of reserve food material begins at this period. Davies (1934) has shown that once the larvae have entered the early stages of the third instar about 70 per cent. can give rise to undersized adults if deprived of food. Thus sufficient reserves must have already been accumulated for metabolism during metamorphosis.

IV. Prepupae and Pupae.

Cousin (1932) has made an intensive study of diapause in the prepupae of *L. sericata* and its effect on later stages. Evans (1932) has studied the chemical changes which occur during metamorphosis.

Prepupae can complete their development over a range of temperatures from 10–37° C. and have a theoretical threshold of 4·2° C. (Wardle, 1930). According to Cousin (1932), they have a theoretical threshold of 9·4° C. The discrepancy between the theoretical temperature values recorded by Cousin and Wardle is very large and admits of no clear explanation from the details

of the experiments as recorded. Wardle records that some deaths occurred in all his experiments and in many the mortality was higher than 70 per cent. Cousin did not experience this mortality in her experiments, and neither have I. The mean lengths of the prepupal stage at various temperatures as recorded by Wardle are much longer than those recorded by Cousin except at 20° C. where the results are similar. In view of the high mortality and the variability in length of the stage of the prepupae studied by Wardle it would appear that his experimental conditions were at fault. Such data as I possess support the figures of Cousin. At 9° centigrade only 10 per cent. of the prepupae pupated (Evans 1935*a*). Between these temperatures and 0° C. the prepupae enter into a resting condition known as diapause, in which they remain until removed to favourable temperatures or else, under constant conditions, until death occurs. Demarcation between the larvae and prepupae is difficult as there is no obvious external morphological criterion other than the presence of food in the crop and alimentary canal indicating that digestion and assimilation are still proceeding. Insects which have left the meat and burrowed in the underlying soil or sand can be regarded as larvae if their crops or alimentary tracts still contain food remains. Cousin has shown that there is a clear-cut difference in the reaction of fully-fed larvae and prepupae to cold. Larvae exposed to — 2° C. for several hours expand, paralytically, and many die; still more die after the temperature has been increased to 30° C., very few pupating. Prepupae 10 hours old, if exposed to the same conditions, react differently; 10–15 per cent. die in expansion, the remainder contract. On removal to 30° C., most pupate, 10–20 per cent. remaining in diapause for 3–15 days. Similarly prepupae are more resistant than larvae to still lower temperatures, larvae being killed after an exposure of a few minutes to — 10° C. while prepupae can withstand this temperature for at least 1 hour.

In *Lucilia* the loss of weight and the intensity of respiration follow the normal course established for several other insects, *i.e. Calliphora erythrocephala*, Weinland (1908), *Ophyra*, Tangl (1909). Loss of weight at 30° C. is very rapid during the first 80 hours of prepupal life and the early part of pupal life, slow for the next 50 hours and increasingly rapid again during the last 70 hours (Table IV).

Table IV also shows some of the data of Cousin on respiration during metamorphosis. The respiratory quotient of larvae during growth oscillates

TABLE IV.

Loss of weight and changes in respiratory activity during metamorphosis at 30–32° C.

Age in hours.	Weight of 100 individuals.	R.Q.	Oxygen consumed per hour per gram.
2	5·02 gms.	0·78	3·12
26	4·69 „	0·70	0·83
45	3·82 „	0·63	0·47
60 (prepupae.)	3·42 „	0·68	1·10
60 (pupae.)	3·33 „	0·62	1·13
108	3·15 „	0·52	0·50
127	3·02 „	0·63	0·45
151	2·90 „	0·69	1·11
200	2·84 „	0·69	1·49

(Cousin, 1932.)

between 0·76 and 0·82, as would be expected from an insect living on a food with a high protein content. On cessation of feeding the R.Q. drops rapidly to about 0·70 and later still further, rising a little just before pupation. The variations in the R.Q. during the pupal period are of little importance (her data are much more extensive than those given above). But it may be noted here that during the last half of the prepupal life and during the whole of pupal life, the R.Q. is consistently below 0·70, rising to 0·81 in emerging flies. The intensity of respiration also falls rapidly in young prepupae, rising again just before pupation. As soon as the chitin of the puparium has taken on its characteristic brown appearance, the consumption of oxygen falls. During the last period of pupal life the intensity of respiration again rises.

The broad chemical changes which occur during metamorphosis have been followed by Evans (1932). Table V shows the loss in weight of protein, fat and carbohydrate during prepupal and pupal life.

TABLE V.

Loss in weight in mg. of various substances from 100 individuals during prepupal and pupal stages, taken from data of Evans (1932).

Stage.	Duration of stage in days.	Protein-N.	Fat.	Carbohydrate as glucose.
Prepupae .	6	24	125	35
Pupae . .	9	76	53	31

Fat and carbohydrate are metabolised more intensely during the prepupal period than during the pupal period, but the reverse is found for protein.

At 17° C. fat is consumed rapidly during the first 10 days, and then a synthesis of fat occurs reaching a maximum on 14–15th day after which time it falls again. Analysis of the fat shows that it is principally the unsaturated fatty acids which are metabolised. The amount of carbohydrate present decreases rapidly until the thirteenth day, after which it remains constant.

The changes in nitrogen distribution were followed in the following fractions :—

(*a*) insoluble protein nitrogen which was regarded as an approximation to the amount of organised tissue present;
(*b*) soluble protein nitrogen representing soluble reserve proteins and products of histolysis;
(*c*) peptones and proteases;
(*d*) Amino-acid nitrogen;
(*e*) excretory nitrogen;
(*f*) chitin nitrogen.

During the first two days of the prepupal period, a synthesis of insoluble nitrogen compounds occurs; this probably means that a certain amount of insoluble reserve protein is included in fraction (*a*) since insoluble protein reserves have been found in the fat-body cells of the newly emerged adult (Evans, 1935*c*). These fat-body cells are left over from the pupa and soon utilised by the adult. The principal change occurring during the prepupal period is a mobilisation of protein nitrogen for synthesis into the chitin of the puparium; insoluble and soluble protein nitrogen fall, while peptone and chitin nitrogen rise.

Insoluble protein continues to fall after pupation until the 9th day, when

it rises rapidly. This rise coincides with the period when histolysis of larval muscle ceases and histogenesis proceeds rapidly (Evans, unpublished). Soluble nitrogen increases from the 6th to the 9th day and then falls rapidly, being utilised by the growing tissues.

The concentration of amino-acids increases until pupation occurs, remains constant until histogenesis begins, and then slowly falls until the fly emerges. The formation of excretory nitrogen is most rapid from the 4th day until the 9th day, and apparently ceases a few days before emergence.

Wigglesworth (1932) suggests that the rectal glands play an important part in reabsorbing water during pupal development. At first the Malpighian tubes are clear and translucent; the hindgut and rectal pouch are empty. Later granules of uric acid appear in the Malpighian tubes and the hindgut and rectum become greatly distended with fluid. The uric acid granules accumulate in the rectum, which becomes filled with them shortly before emergence. It is difficult to account for such observations without assuming a continuous reabsorption of water from the rectum; and such absorption must be ascribed to the rectal glands.

The above is an account of the normal processes occurring during metamorphosis. Roubaud (1922) classifies *L. sericata* as belonging to his "heterodynamic" class of insects, that is after a number of generations a stage of the insect concerned enters into an obligatory diapause independent of environmental factors during which it undergoes a process of rejuvenation. The obligatory diapause is supposed to occur during the prepupal stage of *L. sericata* —the stage in which it normally hibernates. The extensive researches of Cousin (1932) show that there is no obligatory or rhythmic diapause in this species. This investigator was able to rear numerous generations of the insect without any spontaneous obligatory diapause occurring. A diapause can be induced in prepupae by several means: exposure to low or high temperature, placing in very dry or very wet sand, presence of toxic gases such as carbon dioxide and ammonia, and overcrowding of the pupae. The conditions which bring the diapause to an end are generally in direct relation with those which have brought it about, *i.e.* a diapause brought on by dryness breaks in a humid atmosphere. But if the duration of diapause has been long or the causative factors very intense, this direct relation is masked and other factors must be brought in to play the rôle of stimulant. The common factors for breaking diapause are heat, humidity and aeration, and stimulants such as heat, shock and light. Both sexes are equally susceptible to factors inducing diapause, but the male sex seems less resistant to prolonged diapause. Once diapause has been broken, development proceeds as for normal individuals. Practically the only difference which may be observed is the smaller size of adults obtained from prepupae which have remained in diapause for some time.

Cousin has also shown that the numbers of prepupae entering into diapause is in direct relation to an excessive loss of weight at low humidities and to an abnormally low loss of weight at high humidities (Table VI). The percentage dry weight of diapause prepupae kept in dry sand increases progressively.

Normal	26·5–28·6 per cent.
Diapause 2 months	35·6–34·8 per cent.
„ 3½ „	46·7 per cent.

If such prepupae are removed to wet sand, they drink water and rapidly increase in weight; the amount of water absorbed is in direct relation with the "dryness" of the individual.

Table VI.

Loss of weight from 100 normal individuals after exposure to various humidities.

No. in diapause when weighed.	Percentage loss of weight.	Humidity.
78	8·7	90–100% R.H.
22	12·4	progressively drier.
8	15·5	progressively drier.
3	17·6	45–60% R.H.
2	21·3	25–30% R.H.
66	31·0	very dry.
89	44·2	completely dry.

(Cousin, 1932.)

As would be expected the respiratory activity of prepupae in diapause is very low (Table VII). When diapause is broken by suitable stimulants, the consumption of oxygen increases, *i.e.* from 0·34 c.c. to 1·10 c.c. per gram per hour.

Table VII.

Metabolism of prepupae in diapause due to dryness.

R.Q.	C.c. O_2 per gram per hour.	Time.
0·76	0·45	15 days
0·69	0·39	5 weeks
0·70	0·17	3 months

(Cousin, 1932.)

V. The Adult.

The optimum conditions for rearing large numbers of adults have been worked out by Cousin (1932). The principal factors are : size of cage, light, temperature, humidity and food. The cage used must have a volume of at least $\frac{1}{3}$ of a cubic metre, otherwise copulation is interfered with and only sterile eggs are laid. Good light, preferably sunlight, is indispensable, and an electric lamp placed near the cage is a suitable substitute in winter and during dull summer periods. These flies will not copulate in the dark. The temperature of the cage should be between 20° C. and 30° C., and eggs will be laid in about 4–7 days. Suitable humidity conditions are provided if two basins about 9 inches in diameter filled with water are placed in the cage. The basins should be covered with muslin and the water allowed to wet the muslin. Cousin emphasises the necessity of spraying the walls of the cages frequently with water, but I have not found this to be necessary as long as the flies have a suitable source of water for drinking purposes. Nitrogenous substances necessary for the growth of the ovaries are provided by meat, which should be kept moist. Carbohydrate in one form or another is also necessary. Cousin advises soft pulpy fruits, but I have found lump sugar quite satisfactory. Under these conditions the flies should live about 1 month, the females living rather longer than the males.

Development of the reproductive organs in the adult fly differs in the sexes (Cousin, 1932; Mackerras, 1933). In the female it is dependent upon an

adequate diet, whereas in the male a few active sperms may be found in the testes within 12 hours of emergence and the proportion of active sperms increases during the next two days although the flies have had only water to drink. Female flies fed for one day on liver, sugar and water, then mated, laid fertile eggs on fresh liver when introduced on the 7th day. If meat is withheld from the females for more than 10 days, the ovaries fail to develop (Cousin, 1932). These flies lay large numbers of eggs when kept under optimum conditions. Mackerras (1933) records one female as laying at least 2,373 eggs with an average of 190 eggs per batch. Salt (1932) also gives some figures for the number of eggs laid by individual females.

Oviposition stimuli are usually provided by the presence of fresh or decaying animal matter (Mackerras, 1933), but if this is withheld for some days from females ready to lay, they will deposit these eggs on rotten fruits, damp muslin or even on the sides of the cage. Oviposition is independent of fertilisation, unmated flies laying eggs at irregular intervals in the same way as mated flies. In this way *sericata* differs from the closely related *L. cuprina*, in which species unmated females do not lay eggs.

Nicholson (1934) has studied the effect of temperature on the activity of some blow-flies. The experiments were carried out under certain standard conditions and are most interesting from a comparative point of view; the curves of general activity and temperature preference correspond to the known distribution of the four species examined. Two species of *Lucilia* are studied, *L. sericata* and *L. cuprina* Wied., and two species of *Calliphora*. The following is a comparison of the two *Lucilia* species. "The morphological differences between *L. cuprina* and *L. sericata* are so slight that one can distinguish these species with confidence only after much practice yet their temperature relations are quite distinct, the zone of activity of *L. cuprina* being decidedly higher, and its zone of intense activity definitely narrower, than those of *L. sericata*. This lends point to the argument that the differences which have led to the evolution and separation of species may be inconspicuous ones which cannot be recognised by taxonomists when describing species." *L. cuprina* essentially belongs to the warmer regions of Australia and *L. sericata* to the cooler ones. *L. sericata* is most active between 20° C. and 35° C.

It is well known that *L. sericata* is a sun-loving species. Holdaway (1933) suggests that it differs from *L. ceasar* and *L. ampulacea* in that the range of waves of radiant energy favouring the activity of *L. sericata* is different from that favouring the activity of *L. ceasar* and *L. ampulacea*, the two latter species being found principally in shady habitats.

Evans (1935*c*) has studied in some detail the growth processes which occur after emergence. Immediately after emergence the young "walker" swallows air which collects in the mesenteron. This causes the volume of the male to increase by about 17 per cent. and that of the female by about 33 per cent. The abdominal sclerites now harden and the amount of air in the mesenteron begins to diminish, diffusing through the blood to the atmosphere. As the abdominal sclerites are now practically rigid, they cannot collapse to their former wrinkled state and air enters into the flattened anterior abdominal air-sacs through the spiracles. These air-sacs commence to be filled about two hours after emergence and are fully expanded in eight to ten hours at 30° C.

Flies commence to feed fairly soon after emergence and increase in weight rapidly. The males increase in weight rapidly for one day and then, sur-

prisingly, decrease in weight steadily until they die somewhat below their original weight at emergence. The females also increase in weight rapidly for one day gaining roughly the same amount as the males, about 30 per cent. of their original weight. They increase in weight slowly for the next three days and then rapidly for two days, finally weighing about 70 per cent. more than their original weight. During the first period of increase in weight the imaginal fat-body increases in size greatly and during the second the ovaries grow to their full size. The remains of the pupal fat-body are present in the young imago and contain proteid granules. These granules disappear more rapidly in flies fed on growing diets than on non-growing diets so it would seem probable that the granules are quickly made available for the growing tissues.

The forward growth of the imaginal fat-body and ovaries compresses the anterior abdominal air-sacs so that but little air remains in them by the sixth day. It is therefore unlikely that in this insect the function of these abdominal air-sacs is to lower its specific gravity. Neither are they of any especial value in increasing the oxygen supply to the fully developed adult since the rate of metabolism of flies six days old, with compressed air-sacs is about seven times that of unfed flies with fully expanded air-sacs. The hypothesis is put forward that, in flies of the Muscid type, the main function of the anterior abdominal air-sacs is to maintain the increased volume of the young imago and so provide ample space for the growth of the fat-body and ovaries. The fact that the increase in volume of the emerging female is about twice that of the male fits in well with this hypothesis since the male increases in weight by 30 per cent. and the female by 70 per cent.

Some observations on the length of life at several humidities of flies fed on various diets for one and six days are given by Evans (1935*b*). Unfed flies and flies fed for one day die more quickly at low than at high humidities, life being longest at 100 and 80 per cent. relative humidities. Flies fed on growing diets (meat, sugar and water; and sugar and water) live longest, while unfed flies live longer than flies fed on non-growing diets (meat and water; and water alone). These differences can be understood on the following facts, flies fed on growing diets rapidly lay down food reserves while flies fed on non-growing diets lose some of the reserve material present in the newly emerged fly. Also the rate of metabolism is distinctly lower in unfed than in fed flies.

The reaction to humidity of flies fed for six days differs distinctly from that of flies fed for one day. Only flies fed on growing diets are alive on the sixth day. The maximum length of life now occurs at 80 per cent. relative humidity, saturated or nearly saturated air being distinctly injurious. A difference in the length of life of the sexes is now shown, the females living distinctly longer than the males. Also flies fed on sugar and water live much longer than those fed on meat, sugar and water. The length of life of these flies is correlated directly with their carbohydrate content and not with their fat content.

Unfed flies emerging from pupae kept at different humidities live for equal periods of time at 0 and 50 per cent. relative humidity.

A high humidity is necessary for the development of the ovaries. At 23° C. complete development of the ovaries only occurred at 100 and 80 per cent. relative humidity, 95 and 40 per cent. of the flies initially present being alive on the sixth day. At 40 per cent. relative humidity, all the flies were dead by the fourth day with their ovaries one-fourth to one-third developed. All

flies at 0 per cent. relative humidity were dead by the second day. This relation between growth of the ovaries and humidity would appear to be brought about by differences in the rate of metabolism of flies kept at different humidities, the higher rate of metabolism of flies kept at low humidities lessening the amount of material available for growth of the ovaries. Humidity has no effect on oviposition at temperatures of 10–35° C., but at 40° C. low humidity is unfavourable.

The highest temperature which the adult can survive for one hour is 43° C. and is not dependent on the humidity conditions prevailing at the time, Mellanby (1932). The highest temperature which they can survive for twenty-four hours, however, is dependent on humidity, thus at 0 per cent. relative humidity they can survive at 32° C., but at 90 per cent. relative humidity they can survive at 36° C.

MacIndoo (1933–1934) has studied the chemotropic responses of males and females to various substances and shows that males and females do not differ in any appreciable way. Solutions of fermenting sugars are attractive for 18 days, but if baker's yeast is added they are only attractive for 20 hours, after which times respectively they become repellent. Since dilute concentrations of alcohol proved attractive and carbon dioxide and acetic acid were repellent it is inferred that the response obtained depends on the amount of these substances present in the fermenting liquor. Milk, lactic acid, lactose and casein are also attractive, sour milk more so than fresh, probably on account of the increased concentrations of lactic acid. Minnich (1926) claims that chemoreceptors present on the tarsi serve as organs of taste. MacIndoo shows that it is not necessary to assume that the sense organs on the tarsi are gustatory in function, as it is impossible to wet them with water or sugar solution. Also the extension of the proboscis preliminary to feeding can be called forth when the surface of the sugar solution is held about 3 mm. away from the tarsi. Therefore the sense organs on the tarsi would appear to be olfactory in function. He finds no evidence for the presence of olfactory organs on the antennae or palps. Hoskins and Craig (1934) have shown that *L. sericata* is attracted by low concentration of secondary amyl mercaptan but repelled by higher concentrations. Freney (1932) has shown that the products of hydrolysis of keratin, the principal protein of wool, are highly attractive to blow-flies and that the addition of sodium sulphide both increases and prolongs the attractiveness of other baits.

Under normal conditions the flies react to the vapour of ethyl butyrate by an extension of the proboscis and regurgitation of fluid. Abbott (1932) has shown that the percentage of flies responding to the vapour of this substance is highest at 30° C. Humidity also influences the percentage of responses, the highest percentage being found at 73 per cent. relative humidity.

VI. The Physiological Responses of the Adult leading to Fly-strike of Sheep.

Holdaway (1932) is of the opinion that fly-strike or oviposition on living sheep as opposed to carrion is essentially a natural phenomenon due to chemical and physical factors in the fleece and that Froggatt's assumption that the flies have acquired a new habit is not justified. Experiment showed that, on rabbits killed so that no blood was spilt and laid on the ground, oviposition occurred mainly in the fur, especially in the fur which was moist through contact with the ground. The young larvae passed their early life, at least up to the beginning of the second instar, on the surface of the skin. These

observations were confirmed on dead sheep. Attraction of the flies to various substances is due to the presence of products of bacterial activity. These products also provide the stimuli necessary for oviposition. Thus it would be logical to expect that fly-strike is associated with bacterial activity in the fleece. That this is so has now been confirmed. Seddon (1931) has demonstrated the connection between " weather stain or water rot " brought about by bacterial agencies and fly-strike. C. R. Mulhearn and M. S. Mackerras have shown that there is always a high bacterial count in wool prior to strike.

Hobson (1935*d*) has done some further work along these lines, investigating the type of substance which attracts and stimulates oviposition by the female. He finds that although larval excreta attracts blow-flies it does not by itself induce egg-laying. However, when placed on *live* sheep the excreta invariably induced oviposition on warm sunny days. The points of especial interest are (1) only females of *L. sericata* respond, whereas carrion attracts many species; (2) the living sheep plays an essential part. As it is not necessary for the excreta to be placed in direct contact with the wool, it would seem that attraction is due to two sets of factors, one supplied by the sheep and the other by the excreta. Further experiments suggest that any source of ammoniacal decomposition will induce *L. sericata* to oviposit on sheep.

The importance from the point of view of control of the sheep blow-fly of the above type of work is very great. Investigators now have a reliable means of inducing experimental strike for testing repellents under field conditions without being dependent on the vagaries of climate to produce a sufficiency of strikes at convenient times and places. It also has an important bearing on attempts to lower field populations by trapping, as it is possible to catch only *L. sericata* and not the other blow-flies which compete with it for available food, thus intensifying natural competition.

VII. Summary.

The effect of temperature and humidity on all stages in the life-history of the insect is discussed.

Micro-organisms play no part in the digestion of food in the gut of the larvae, their importance lies in the synthesis of an accessory growth factor and the liquefaction of the food. Enzymes acting upon carbohydrates are absent except for a weak amylase in the salivary glands; proteolytic and lipolytic enzymes are active in the midgut, the former being present also in the excreta. The accessory growth factors consist of cholesterol, vitamin B and three other factors probably analogous to members of the vitamin B complex. Photoreceptive organs are present on the bilobed larval maxillae.

The chemical changes and gaseous exchanges occurring during metamorphosis are described.

In the adult, the function of the large anterior abdominal air-sacs is to maintain its increased volume after emergence and so provide room for growth of the fat-body and ovaries. The age and diet of flies are important factors in their reaction to humidity.

The attraction or repulsion of decomposing matter for flies seems to depend on the relative amounts present of certain substances such as alcohol, lactic acid, carbon dioxide, acetic acid, etc. The olfactory organs appear to be present on the tarsi and not on the antennae or the palpi.

Bacterial activity in the fleece is the chief cause of flies ovipositing on living sheep. Two sets of factors are involved, one set provided by ammoniacal products of bacterial activity and another provided by the living sheep.

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